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(54)【発明の名称】　コンプレックス多酵素貯蔵安定性反応混合物の製造方法とその使用方法

(57)【要約】
ａ）反応媒質剤、補助因子、基質を持つ自然のあるいは人工的な酵素による活性タンパク質混合物が貯蔵安定性を持つよう準備され、その際使用者固有の鍵コンポーネント（例えばｍＲＮＡ）だけが、望ましい酵素による１つあるいは複数の反応のスタートのために欠けている。溶液中の反応混合物に、一方では多酵素システムの反応能力を高め、他方では不安定な反応コンポーネントの、貯蔵の安定化および貯蔵の間の生物学的活性や生物学的に活性の構造の損失から保護する安定剤が添加される；ｂ）反応混合物は真空での単純な凍結乾燥により貯蔵安定にされ、その後４－１０℃（冷蔵庫温度）で持続的に貯蔵できる；ｃ）使用者は使用前に、使用準備の出来た反応混合物にもとの量のH_2Oを付加するだけで再構成しなければならず、望ましい酵素による１つあるいは複数の反応が使用者に固有な１つあるいは複数の鍵コンポーネントにより開始される。

1.This document has been translated by computer. So the translation may not reflect the original precisely.
2.**** shows the word which can not be translated.
3.In the drawings, any words are not translated.

CLAIMS

[Claim(s)]
1. On the Other Hand, Heighten Reaction Capacity of Multi-Enzyme System with Reaction KOMPONENNTO. The stabilizer which protects the biological activity or biological activity structure of an unstable reaction component during storage stabilization and storage on the other hand is included. Combine in the solution of water solubility [mixture / artificial / enzyme activity / nature or / protein]. Then, it is characterized by changing to the condition that there is storage stability at 10 degrees C from 0 degree with freeze drying. It is the manufacture approach which is the manufacture approach of the reaction mixture of complex multi-enzyme storage stability, and is characterized by being equal to a complement in order that the amount of the stabilizer which raises the activity by the enzyme of a reaction mixture in that case may stabilize storage of complex multi-enzyme reaction mixture.
2. Approach according to claim 1 characterized by using cell extract, cell melts, or these fragments as natural enzyme activity protein mixture.
3. Each enzyme which the different origin depending on case refined beforehand as artificial enzyme activity protein mixture, cofactor, approach according to claim 1 characterized by using combination of structural protein depending on case.
4. as a reaction component -- an enzyme and non-enzyme cofactor, an enzyme substrate, a nucleotide, nucleosides or these oligomer, protein, a peptide, thiol compounds, RNA and DNA, and a case -- the derivative of all the above-mentioned matter -- each -- or an approach given in the 1st term in claims 1-3 characterized by combining and using it.
5. Approach given in the 1st term in claims 1-4 to which stabilizer is characterized by being sugar of 8 to 12 in water-soluble solution% (M/Vol) of concentration.
6. Approach according to claim 5 characterized by sugar being TEREHA sirloin.
7. Approach given in the 1st term in claims 1-6 to which vacuum drying of multi-enzyme reaction mixture is characterized by being carried out from 3 for 4 hours at room temperature in commercial freeze-drying equipment.
8. Approach according to claim 7 characterized by freezing reaction mixture in dry ice / alcoholic bath just before vacuum drying depending on case among liquefied nitrogen.
9. Use which is use of one complex multi-enzyme storage stability reaction mixture in claims 1-8 for protein after underwater reconstruction or composition of nucleic acid, qualification, or analysis, and is characterized by adding one or more specific key components each time in that case.
10. Use according to claim 9 characterized by a key component being the combination of DNA in which it has the derivative and oligomer, nature or artificial messenger RNA, and the different origin depending on radioactivity, nonradioactive indicator amino acid or its aminoacylated corresponding tRNA molecule, radioactivity, a nonradioactive indicator nucleic acid, or the case, or the above-mentioned matter.
11. Use given [with the case for an acellular ribosomal protein biosynthesis] in claim 9 for the posttranslational modification of a peptide, a polypeptide, and protein, or any 1 term of 10.

12. Use given [with a case] in a duplicate after concentration of the nucleic acid in a test tube, or qualification, claim 9 for a reversible or irreversible imprint, or any 1 term of 10.

[Translation done.]
DETAILED DESCRIPTION

[Detailed Description of the Invention]
The manufacture approach and operation explanation of a complex multi-enzyme storage-stability reaction mixture This invention can be used immediately and relates to the manufacture approach of the complex multi-enzyme stabilization reaction mixture which is nature or consists of artificial enzyme activity protein mixture depending on the case which stores that there is completely no loss of activity at refrigerator temperature (from 0 degree to 10 degrees C), and can be conveyed, the approach for composition of a polypeptide and a nucleic acid, qualification, or analysis, and use.
The complex reaction system use which consists of the cell extracts and enzyme mixture for research of a biological reactivity becomes increasingly important in modern biology, and the medical diagnosis is also important.
The problem of target-izing of proteinic composition, folding, the maturation after a translation, and intracellular protein is studied by the cell-free extract and melt which are equipped with the equipment of the perfect ribosome for protein biosynthesis quite from before. Multi-enzyme reaction mixture [as opposed to acellular protein biosynthesis (especially translation within a test tube) besides use by the fundamental research] is more important for solution of the target of preparation-composition still. The translation in a test tube was further used for composition of the protein part for production of a main antigenic determinant, catalytic center, or a substrate formation part. The need to the tumor diagnosis of the reaction mixture which the handling for the translation in the test tube of Composition mRNA is easy, and standardizes, and can be used immediately generated through RT-PCR is increasing since the so-called installation of the protein cutting assay (PTU) for related genome variation detection.
It is the description that other analytic molecule biological methods like the RNA biosynthesis (imprint reaction in a test tube) in polymerase chain reaction (PCR), DNA sequencing, and a test tube replace one conventional enzyme assay with a multi-enzyme reaction system. The function which specialized two or more enzymes and cofactors, a joint ** cell, and processing go up, and the mutation rate of the DNA and RNA polymerase in a test tube decreases. it is essentially alike, a long DNA fragment (>10kb) is amplified, and it gets rather than it is based only on one enzyme as a result.
Composite accuracy and the yield of a product rise more and its composition in which the nonspecific by-product made a mistake decreases more. As the enzyme combined with a multi-enzyme reaction system in PCR, and a protein factor, DNA polymerase with an inorganic pyrophosphatase, DNA-binding protein, a polymerase specific antibody, and different exonuclease activity is mentioned.
With the current technical level, use of multi-enzyme reaction mixture was dealt with, there are a series of faults about repeatability and storage stability, and use of the multi-enzyme reaction mixture for the research applied, and the protein and DNA synthesis in a diagnosis is delayed.
A limitation is in complicated handling and storage stability, or the decisive demerit of a multi-enzyme reaction system over single enzyme assay is are unstable. As a water-soluble solution, if the perfect reaction mixture with all enzymes, a substrate, and cofactor carries out long duration

progress, it will not be stabilized in the condition of having made it a room temperature or freezing, either. This is because it has deviated from the optimal conditions (a pH value, ionic strength, the concentration of an enzyme and a stabilizer, class of salt) for an enzyme component, preservation of cofactor, and implementation of a biochemical reaction required for continuous stability. (The process of Franks and F. (1989) biotechnology chemistry, 24(1) R3-R7) .
With multi-enzyme reaction mixture, the problem of preparing the demand from which each component of a cell extract or enzyme mixture differs to a stabilization medium and a storage condition by whether they being soluble ferment, fibrous structural protein, the film, the enzyme complex that met, or a nucleoprotein is added. In many cases, various demands do not compromise mutually. For this reason, the reaction system of marketing for the translation in a test tube, an imprint, PCR, or DNA sequencing is offered in the form of a kit, and a component is separately prepared in this. It dissociates and the component with which kits differ is stored on different conditions as the following examples of the reaction mixture for the translation in a test tube show.

反応混合物のコンポーネント	濃度ファクター	貯蔵温度
1．マスター混合物（HEPES－KOH，ATP，GTP，DTT，tRNA，スペリディン（Sperdin）、リン酸クレアチン）	12．5X	－20℃
2．アミノ酸混合物（全てのアミノ酸の2．5mM）	50X	－20℃
3．クレアチンキナーゼ	25X	4℃
4．RNaseインヒビター	－　－　－	－20℃
5．細胞抽出物／溶解物（無細胞抽出物、K－酢酸塩、Mg－酢酸塩、HEPES－KOH，DTT）	3X	－80℃
6．翻訳緩衝剤（K－酢酸塩／Mg酢酸塩）	25X	－20℃

A user is to blame for always mixing a reaction addition product with each component before experiment initiation. It is difficult for this activity step to have a common mistake and to automate. Time amount becomes long with the number of parallel reactions. According to this prerequisite, preparation for an experiment takes time amount from experiment execution from the first in many cases.
The technical time and effort for storage of a start (4-degree-C, -20-degree-C, -80 degrees C) component and conveyance by various temperature demands is high according to this. Although assembled for the translation in a test tube, migration must be performed in the condition of having dried, in order to avoid that a cell extract melts.

While it is sensitive, it is the cell extract which is translation analysis in a test tube and with which a key component has the nucleoprotein complex of the macromolecule for the protein synthesis of ribosome. Transition of the complicated biochemical reaction of RNA control protein composition needs the interaction of different structure, many enzymes with stability, enzyme complex, and structural protein. Unlike intracellular [which is stabilized by the interaction in which the protein complex of the macromol of ribosome translation equipment has the filament of a cytoskeleton], a cell-free extract and a melt (Lysate) do not contain a perfect cytoskeleton. It dissociates in a solution freely simply and, thereby, the complex of a macromolecule loses reaction capacity.

The approach uniquely permitted biologically [fusibility] until now for preservation of the cell extract of activity was storage in the condition of having frozen at low temperature at -80 degrees C / -120 degrees C. Thereby, a series of problems and faults arise. The cell extract and melt which consist of a germ of wheat, reticulocyte, and a bacterial cell when it freezes lose activity with an original enzyme, because it is because much protein is irreversibly injured by formation of the crystal of water. Then, if it is made to freeze numbers of times and melts for an experiment, in the acellular melt and reticulocyte which consist of E.coli(s), translational activity will be lost nearly completely. In a wheat germ extract, activity loss is about 20 to 40% after all dissolutions and freezings.

The instability of a cell-free extract to freezing and the dissolution of a repeat brings a user uneconomical use. In order to attain a reproducible experimental result, all the preparations of a translational activity cell extract must be melted once again. The possible substituting method is the storage of the prepared reaction mixture which was [application] completely ready frozen at ** and -80-degree C low temperature. The test by the translation analysis which used the wheat germ extract as the base shows that translational activity is lost 60% compared with freezing of -80-degree C once, and the control reaction already produced newly in the storage for one week. Although the acellular protein extract condensed by this is storage stability comparatively also at low temperature, it is not so in a reaction mixture with the diluted cell extract. A decisive element for this is the high protein concentration in the cell extract with a stabilization effect which has not been diluted.

The problem of the stability in the reaction mixture for the imprint reaction in PCR and a test tube and DNA sequencing is the same. The freezing in the condensed water-soluble solution itself makes DNA and RNA polymerase inactive completely, and only when there is a high-concentration low-temperature protector at -20 degrees C as a result, it becomes activity. Usually, by high concentration, the glycerol (50%) used as a low-temperature protector and other things (DMSO, polyethylene glycol) injure the translation in PCR (formation of primary annealing), and a test tube (KUROVE, L).

M., J.H. KUROVE, Dev.Biol.Stand.285-294. In 50% or less of glycerol concentration, the storage stability of an enzyme falls at -20 degrees C.

Preparation of the cell-free extract for the translation in the test tube of the middle of the 70s is not changing the approach for manufacture and the stable storage since the first announcement. The method of substituting for the above-mentioned advanced technology will be stabilizing storage with freeze drying. This approach has a rich result, without using an enzyme component for the stability of liposome, a film fragment, each enzyme preparation object, or a partial reaction mixture, and the special sugar or the polyol combined with the metal ion of bivalence or the surfactant at that time prevents or restricts deterioration of a living thing molecule by loss of water.

The sugar trehalose combined with the low-temperature protector of common knowledge like PEG or DMSO is suitable for especially the storage stability of an enzyme preparation object (19.9 10). This sugar is a natural metabolism product of many vegetation, an insect, and a microorganism which is accumulated by intracellular on certain stress conditions (a heat shock, a dehydrogenation, radiation), and secures that this living thing survives.
The technical issue used as the foundation of this invention is offer of the manufacture approach of removing the fault of the advanced technology about the reaction capacity of the multi-enzyme reaction mixture of the complex for execution of reaction transition of biochemistry, storage stability, a translation, and application preparation. The reaction mixture stored and translated at low temperature is not used, but the reaction mixtures (that is, it had all the reaction components) which were [application] ready decrease in number a user's experiment costs remarkably, and especially this approach draws a product which raises repeatability.
A technical problem is solved by claims 1 and 9 with this invention. A subordination claim is related with a special operation gestalt.
on the other hand , the approach by this invention have the description in activity protein mixture be combine in a water-soluble solution with nature or an artificial enzyme with the reaction component which prevent biological activity or lose the structure of activity biologically , and a stabilizer during storage again , while the reaction capacity of a multi-enzyme system be heighten and an unstable reaction component carry out storage to stability on the other hand . then, this -- freeze drying -- 0 to 10 degrees C -- storage -- it shifts to a stable condition and the amount of the cell stabilizer which raises the activity by the enzyme of a reaction mixture is equal to a complement to the storage stability of complex multi-enzyme reaction mixture in that case.
A cell melt or the fragment of this is used as a cell extract of the protein mixture of activity with a natural enzyme.
The combination of structural protein is used depending on one enzyme by which various origins were beforehand purified as protein mixture of activity with the artificial enzyme depending on the case, cofactor, and the case.
The reaction components of this invention are all the derivatives of each or the combined above-mentioned matter again depending on the cofactor by the enzyme by the enzyme, an enzyme substrate, a nucleotide, a nucleoside or its oligomer, protein, pre PUCHIDO (Preptid), thiol compounds, RNA and DNA, and the case.
As a stabilizer, it is 8-12% (M/Vol) in the reaction mixture which was [application] ready of concentration, the conditions optimal for stabilization of an unstable reaction component are made, the sugar which guarantees the maximum proper product yield of the multi-enzyme reaction mixture under composition further is mainly used, and sugar is mainly trehalose.
In commercial freeze-drying equipment, it is a room temperature, the vacuum drying of multi-enzyme reaction mixture is performed from 3 for 4 hours, and a reaction mixture is frozen in liquefied nitrogen, or a dryer / alcoholic bath just before a vacuum drying by the case depending on the case in that case.
The approach by this invention is related mainly with a wheat germ extract and the multi-enzyme reaction mixture for the translation in the test tube in PCR. The reaction mixture of 50microl was used and freeze-dried by the approach (example 1) of this invention. After various storage time amount, the dried reaction mixture was underwater, depending on suitable mRNA and a suitable case, had radioactive amino acid added and was reconfigurated. Thus, the reaction capacity of the reaction mixture treated and stored is; dyhydrofolic-acid Jupiter-VIII KUTA ** (DHFR, 17)

proved by the translation of various mRNA(s).
They are 5kd(s) and OBERIN (Obelin) (20kD). Quantitive certification of a translation product was performed by the measurement of the radioactive protein with which TCA precipitated which consists of reaction precipitate of 5microl, or measurement of the activity by the enzyme of DHFR in the reaction precipitate of 10microl after keeping it warm at 25 degrees C for 2 hours. Quantitive certification of a translation product was continuously performed by gel electrophoresis in radioautography in SDS-PAG. In order to measure a stabilization student's effectiveness, the translation yield in the reconfigurated freeze-drying reaction mixture was nothing respectively, and the generation yield in the reaction precipitate which is not operated compared storage with those with trehalose. The translational activity of the reaction mixture reconfigurated after storing for one to three months at 4 degrees C is changed among 92 to 100 degrees C compared with the activity in the control reaction by 10% of trehalose which is not operated (drawing 6).
The reaction capacity of the PCR reaction mixture with artificial enzyme mixture which it was reconfigurated and was freeze-dried was inspected in RAPD-PCR assay. The artificial enzyme mixture for PCR was constituted by a Tag-DNA-polymer, trademark registration deep vent-DNA polymerase, and the inorganic Tth pyrophosphatase (a mixing ratio is 10:1:0.2) (unit).
The approach of this invention enables application preparation of the protein mixture of activity with nature with a reaction buffer, cofactor, and a substrate, and an artificial enzyme, and is with this approach. : Activity or the stabilizer which prevents loss of activity structure biologically while, raise the reaction capacity of a multi-enzyme system to the reaction mixture in the solution of - water solubility on the other hand and carry out storage of an unstable reaction component to stability on the other hand is add.
- The vacuum drying of the mixture is carried out in liquefied nitrogen after freezing.
- Cover in a layer with inert gas depending on the case.
thus, the gained storage -- a stable reaction mixture is used for application by supply of the key component of a proper according to a desirable enzyme reaction for composition of protein, a polypeptide, and a nucleic acid, correction, or analysis, after reconfigurating by this invention in the inside of water (1/quality of 1000-Q-).
The reaction mixture manufactured by this invention has the advantage that it is stable and can translate by 0 to 10 degrees C storage. Thereby, in the conventional advanced technology, there are no high costs technically for the indispensable purchase of a low-temperature cooling system, and maintenance, and it ends. The reaction mixture which made [application] that second it had all required components is made, and, as a result, an application person is being able to start a desirable reaction only by adding two key components by one or max. This removes the fault of the advanced technology in respect of the following. : Execution of the coincidence of an parallel experiment of - large number (epitope measurement), Reappearance possibility of the enzyme reaction which carries out - parallel and continues The minimum time amount for - preparation for an experiment Preparation of the reaction mixture of complex of which - preparation was done is exact. Automation of biotechnology chemical reaction transition of the complex in - analytic scale This invention When trehalose is a dehydrogenation, let discovery of raising the product harvest of the proper in the translation in a wheat germ extract and the test tube by PCR be the base besides a well-known protective action. It is not controlled, namely, the generation crop (synthetic protein for every mRNA used) of a proper increases in the translation reaction which newly precipitated compared with reaction precipitate without trehalose depending on trehalose concentration. The maximum generation crop is attained by 10% of w/v (drawing 1).

About a series of model protein and various wheat germ extracts, the importance of the "enhancer" effectiveness of the trehalose in a water-soluble solution can be accepted (drawing 2 a, 2b), and the universal phenomenon for which it does not depend on a product as a result poses a problem.
The unique property of trehalose was newly discovered. With the advanced technology, all the investigated adjuvants of the others used as an effective low-temperature protector or a stabilizer like trehalose for a vacuum drying prevent the translation in the test tube in the indispensable concentration for storage stability (drawing 3).
Furthermore, it became clear that the optimal trehalose concentration for the storage stability by freeze drying was equal to the concentration highest condition in the translation reaction in a water-soluble solution (drawing 4).
The "enhancer" effectiveness of trehalose was discovered also in PCR application.
Like the phone yak in a test tube, if the concentration of trehalose goes up, the crop of an escape of one or more propers will go up, and the escape of the DNA fragment which is not special will be oppressed (drawing 5 a/5b).
Especially a dramatic thing is the trehalose effectiveness in a water-soluble solution at the time of being the escape of the DNA fragment of 10 or less kbs in the Tris-Hcl reaction buffer to which a special product is not extended, when there is no trehalose (drawing 5 c).
Therefore, this invention differs from the advanced technology for using trehalose as a stabilizer in respect of the following. The reaction mixture which was [such perfect many enzymes that it is surprised / application] ready by the approach of this invention at first is generable [that there is no loss of activity in storage stability again] without an enzyme by what is not the enzyme or partial reaction mixture which does not come out only one with two or more unstable protein components. The second is optimizing so that the activity of the multi-enzyme reaction mixture which applied the newly [trehalose] discovered "enhancer" effectiveness, guaranteed storage stability (long period of time) with trehalose sufficient as an only adjuvant, and was reconfigurated by coincidence in the conditions in the approach of this invention may be raised.
In the result of application of the approach of this invention, the reaction capacity of the reconfigurated reaction mixture is higher than the new reaction precipitate without a stabilizer which is not controlled. Unlike the well-known approach, the reaction mixture which carried out the freezing vacuum drying of the third has storage stability without loss of activity for at least six months only in a 0 to 10 degrees C temperature field. Storage at the temperature of 15 degrees C or more and 0 degree C or less causes perfect activity loss within one month.
Below, this invention is explained based on two examples.
1st example: Use as the base the wheat germ extract which used the trehalose of a stabilizer. Generation of the reaction mixture of translational activity with storage stability Generation of the reaction mixture which was [application] ready for consisting of components of a each A reaction mixture On the ice (zero to 4 degree C) in the 2.0ml sterilized micro centrifugal separation machine (a screw plug with rubber packing and an even floor is important), it is moved from each following components with a pipet. :

順序	コンポーネントと組成	体積
1.	1／1000－Q－H_2O	13μl
2.	アミノ酸混合物（20のアミノ酸の全てで2．5mM）	2μl
3.	マスター混合物（312mM HEPES－KOH pH7．6、12．5mM ATP、1．25mM GTP、100mM リン酸クレアチン、625μg／ml イースト tRNA、3．125mM スペルミジン、25mM DTT）	4μl
4.	クレアチンフォスフォキナーゼ 1．4mg／ml	2μl
5.	1M 酢酸カリウム	2μl
6.	25mM 酢酸Mg	1μl
7.	50%トレハロース	10μl
8.	小麦胚芽抽出物（90－1000OD_{260}）	16μl

The component in a reaction container is mixed carefully (don't become a whorl).
The volume of the reaction precipitate completed before carrying out storage to stability is 50microl. The volume of each component must be proportionally changed to the precipitate of 25 or 100microl.
b) Storage stabilization of the translation mixture by freeze drying (freeze-drying) Shock freezing is carried out in liquefied nitrogen after mixing (-120 degrees C), and the 2.0ml open reaction container containing translation mixture is kept warm in liquefied nitrogen for 5 minutes. The reaction mixture frozen after that is moved to the freeze-drying room connected to the commercial vacuum pump as early as possible. Shortly after it has already operated the vacuum pump 30 minutes to 60 a minute, therefore a bulb opens, a strong vacuum will be made at a freeze-drying room. In this invention person's laboratory, the freeze-drying equipment of HETORABU (Heto-Lab) was used. A reaction mixture is freeze-dried at a room temperature (20 to 30 degree C) for 3 to 4 hours. A vacuum chamber is carefully ventilated with inert gas after termination of freeze-drying depending on perimeter air or the case. The entry of air is equipped with a sterilization filter in order to avoid contamination by the microorganism of the freeze-dried reaction mixture. Where a reaction container is sterilized after that, air is intercepted and thrust, and it shuts with a plug, and seals by paraffin. By the case, glass ampul is used as a container for freeze drying, and ampul is heat-sealed appropriately after that.
The reaction mixture freeze-dried in this condition intercepts light, and is stored in a refrigerator at zero to 4 degree C.
c) Translation in reconstruction and a test tube The freeze-dried reaction mixture is placed on ice, and dissolves in 1/1000QH2O of 48microl. The dried remnants are immediately dissolved in water. The mRNA solution of 2microl is added after that (0.5-2micro g/mu l). Reaction precipitate is mixed after taking and moving with a pipet carefully. The reconfigurated reaction precipitate is kept warm at 2 to 25 degrees C for 3 hours for the translation in a test tube. In the radioactive check by the L-[14C]-leucine (or 25S-methionine) of a translation product, reconstruction is performed in the leucine solution of 1/1000-Q-H2O of 44microl, and mRNA of 2microl and 4microl. The amount of the translation product after above-mentioned incubation time amount and in the reaction settlings of 5 or 10microl is determined. decision -- an enzyme -- or (DHFR enzyme activity) it is performed by measurement of the radioactivity (unit cpm) in which the acid precipitate in the measurement container by standard pro SEJUA is possible.

2nd example: Long-Range and a Taq-DNA polymer, A RAPD-PCR which uses as the base Pfu-DNA polymerase and enzyme mixture which consists of pyrophosphatases of sum cell MOFIRUSU sake, Generation of a reaction mixture with storage stability Generation of the reaction mixture which was [application] ready for consisting of components of a each A reaction mixture On ice (from 0 degree C to 4 degrees C), 0.5ml which was doubled with suitable thermostat SAIKURA (Thermocycler) which consists of the following KOMPONNETO and which sterilized is moved with a pipet. :

順序	コンポーネントと組成	体積
1.	1／1000－Q－H_2O	30μl
2.	10X 反応緩衝剤（500mM トリシン－KOH pH9.2 160mM $(NH_4)_2SO_4$, 0.1% のTween 20）	5μl
3.	50X dNTP混合物（12.5mM dATP, dGTF, dCTP, dTTP）	1μl
4.	50mM $MgCl_2$	1.5μl
5.	フォワード プライマー（10pmol／μl）	1μl
6.	リバース プライマー（10pmol／μl）	1μl
7.	50%トレハロース	10μl
8.	ゼラチン（20mg／ml）	0.5μl

Centrifugal separation of the component in a reaction container is mixed and carried out. The volume of the completed reaction precipitate is front 50microl of storage stabilization. A key component is suitable template DNA because of the start of PCR. The volume of each component must be proportionally changed to the precipitate of 25 to 100microl.
b) Storage stability of the PCR mixture by freeze drying (freeze-drying) Example 1b is followed.
c Reconstruction and PCR The freeze-dried reaction mixture is put on ice, and dissolves in 1/1000-Q-H2O of 48microl. The dried remnants are immediately dissolved in water. The template DNA solution of 2microl is added after that (5-50 ng/mu l). It takes and moves with a pipet carefully and reaction precipitate is mixed. The reconfigurated reaction precipitate moves to thermostat SAIKURA (94 degrees C) heated beforehand directly from an ice bath. The denaturation step for 2 to 4 minutes is performed, and it starts according to two PCR programs after that.;
RAPD-PCR: 94 degree C 20 seconds Long-Range PCR:94 degree C 10 seconds 37 degrees C 30 seconds 65 degrees C 20 seconds 72 degrees C 60 seconds 68 degrees C 10 minutes 35 cycle 25 cycle 1. -- after program termination, the reaction mixture of 10microl is put on 0.8% of TAE agar gel, and is analyzed by electrophoresis, respectively.
Description of drawing Drawing 1 The time of the maximum product are recording tested by various wheat germs, the wheat germ extract of DHFR composition crop a high activity of the reaction mixture which is not operated in various trehalose concentration (Lysat SL --) A rise of trehalose concentration settles the DHFR translation reaction (50microl.2microg DHFR-mRNA) of two series by using the cell-free extract (thing of Lysat JB and the shape of a black beam) from a wheat germ with low thing of the shape of a white beam and activity as the base. The maximum density of a product was reached in reaction precipitate after the synthetic persistence time of the test tube introversion translation of 25-degree C 2 hours (refer to translatory motion

of drawing 6 a). A generation crop (DHFR activity) will go up, if trehalose concentration increases, and it serves as max at 10% (M/volume). It becomes the optimal trehalose concentration at 10% in spite of various translational activity for two wheat germ extracts. In Lysat of lower activity, a concentration (12.5% and 15%) dependency is changed for higher trehalose concentration.

drawing 2 a [ ] the "enhancer" effectiveness of trehalose in the translation in the test tube of various mRNA(s) in the reaction mixture which is not operated Measurement of the generation crop under translation reaction of 50microl by three selected mRNA(s) without those with trehalose (radioactivity to which an acid can precipitate). For characterization of the radioactivity of a translation product, the ["S]-methionine (15pmol=349.440cpm) of 1microl was used for every reaction, respectively. The characterized translation product to which an acid can precipitate was taken from the reaction mixture 2 hours after. The following model protein was investigated. : - human being's calcitonin - OBERIN (700bp, 0.25microg)
- E.coliDHFR (500bp, 0.25microg)

The translation reaction by the ["S]-methionine was performed by the RNA concentration below criticality. By higher RNA concentration, composition already reached after the incubation for 15 minutes at the saturation point with the methionine concentration to which it was restricted in reaction sediment.

drawing 2 b [ ] demonstration of the radioactive translation assay by the ["C]-leucine of various model protein which is not controlled Comparison of the generation crop under those with trehalose, and nothing translation reaction of 50microl (radioactivity to which an acid can precipitate). For analysis of the radioactivity of a translation product, the [14C]-leucine (624pmol=349.440cpm) of 4microl was used for every reaction, respectively. For measurement of the translation product analyzed possible [acid precipitate], 5microl was taken from the 3 hours after reaction mixture. :man elongation factor 2 (hEF 2,300bp, 2.0microg RNA), the oligomer structure (SEKUROPIN A-7-mer, 2) of SEKUROPIN A of the peptide of anti bacteria with which the following model protein was compared
5microg RNA, OBERIN (700bp, 2.0microg RNA), E.coliDHFR (500bp, 1.5microg RNA).

Drawing 3 Comparison of an operation of the stabilizer of the common knowledge to the DHFR test tube introversion translation in the reaction mixture of freeze drying which is not controlled and which was reconfigurated again The DHFR translation reaction of two series was performed by the concentration of the sugar from which m/vol was chosen 10%, respectively on standard conditions (DHFR-mRNA of 50microl and 2microg, 2 hours, 25 degrees C). The series (thing of the shape of a white beam) of the beginning of a translation reaction consisted of reaction mixtures which mixed each component just before the start of composition in a test tube and which are not controlled. For the second experiment series (thing of the shape of a black beam), it is freeze-dried first, and is the bidet strike (Bidest) of 48microl after that because of composition.

The perfect reaction mixture reconfigurated underwater was generated. For control for the comparison of a translation crop (DHFR activity), the DHFR translation reaction in a standard reaction mixture was performed without addition of sugar. DHFR activity was measured with the reaction precipitate of 10microl, respectively.

Drawing 4 The comparison of the DHFR composition crop in the reconfigurated translation reaction mixture with the trehalose of various concentration. Measurement of the optimal trehalose concentration for storage stability The translation product which had radioactivity with the acid precipitate capacity of a peak analyzed was attained after the synthetic persistence time

of 25-degree C 3 hours (refer to translation kinematics of drawing 3). The greatest generation crop was attained by 10% (M/Vol) of trehalose like [in the experiment of precedence]. as compared with a control reaction (the translation mixture without trehalose which is not operated -- it is not freeze-drying), reduction of the translation crop in 10% or less of trehalose concentration is based on stability with an inadequate wheat germ [translational activity / under freeze drying].

drawing 5 a:RAPD-PSR effect of trehalose to the performance of assay RAPD-PCR by the accidental primer of the component of ten in the reaction mixture containing the trehalose of various concentration which is not controlled, and DNA of an insect. RAPD-PCR is standard conditions and is AESHUNA. It was performed by the gene DNA of 250ng(s) from SHINEA. Addition of trehalose will strengthen the escape of the DNA fragment (>2kb) of a longer polymorphism, if the background which was made by the wrong escape and which is not peculiar is decreased and concentration rises.

Trace 1 : Standard PCR buffer [50mM Tris - HCl At (25 degree C, pH8.3)-50mMKCl-0.1% triton RAPD-PCR in 100 Trace 2: Tris -- RAPD-PCR in the PCR buffer II [50mM tris-HCl(it is pH8.8 at 25 degrees C)-16mM(NH4)2SO4-0.01%Tween 20] Trace 3-5:2.5%, 5%, The trehalose of v/w 10% RAPD-PCR in the included tris-PCR buffer II Trace 6:DNA 1Kb Ladder Drawing 5 b: The trehalose effectiveness in the PCR reaction mixture which is not controlled Escape of the fragment of 98bp(s) which consist of annular fibrous genes in standard conditions. Addition of the trehalose to the reaction precipitate which is not controlled decreases the background which is not peculiar depending on concentration (wrong escape of 2 or less kbs).

Trace 1:DNA 1Kb ladder Trace 2 : PCR in a standard reaction mixture without trehalose PCR which added the trehalose of 3:5% of traces PCR which added the trehalose of 4:10% of traces Drawing 5 c: Effectiveness of the trehalose to PCR of Long-Range in a tris-HCl reaction buffer Without those with the trehalose which used artificial enzyme mixture Various tris - Long-Range-PCR of 1 DNA fragment of 20kb in the reaction mixture containing HCl which is not operated (Taq-DNA polymerase, Pfu-DNA polymerase, inorganic PIROFOSUTAFAZE). The trehalose of 10%w/v is indispensable and are sufficient conditions for the escape of the DNA fragment of the proper in above-mentioned conditions.

LR-PCR in a Trace 1:standard PCR buffer (tris-HCl/KCl / triton X100 pH8.3) Trace 2:tris-HCl/(NH4)2SO4/Tween LR-PCR in 20 (pH8.8) Trace 3-5: LR-PCR in a commercial tris-HCl reaction buffer Trace 6: A macromolecule sets a DNA marker to 9-48kb (GIBCO BRL). Although it is the same reaction precipitate as a trace 1-5, Trace 7-11 : 10% of trehalose It contains. Trace 12:DNA 1Kb Ladder (GIBCO BRL)

Drawing 6 Experiment concerning stability for a long time by freeze-drying translation assay The perfect translation reaction mixture of 50microl was freeze-dried on condition that common knowledge with all the components that attain to even DHFR-mRNA, light was intercepted, and it was stored at two different temperature. The translation reaction was performed on standard conditions after different time amount progress (50microl, 2microg DHFR-mRNA, 2 hours, 25 degrees C), and DHFR activity was measured. In order to measure only the storage temperature of the translational activity of the reaction mixture freeze-dried for the translation reaction, and the dependency to time amount, the always same mRNA dispensing was used.

DRAWINGS

[Drawing 1]

図1

[Drawing 2]

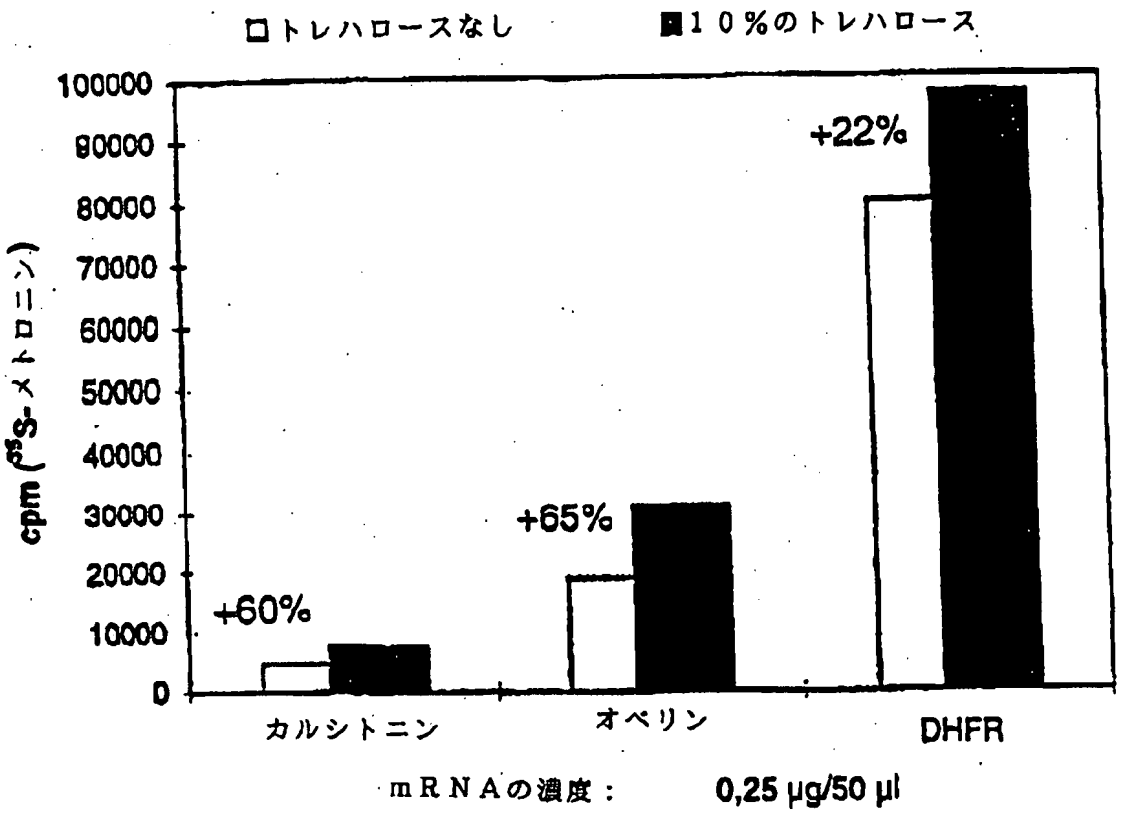


図２ａ

[Drawing 2]

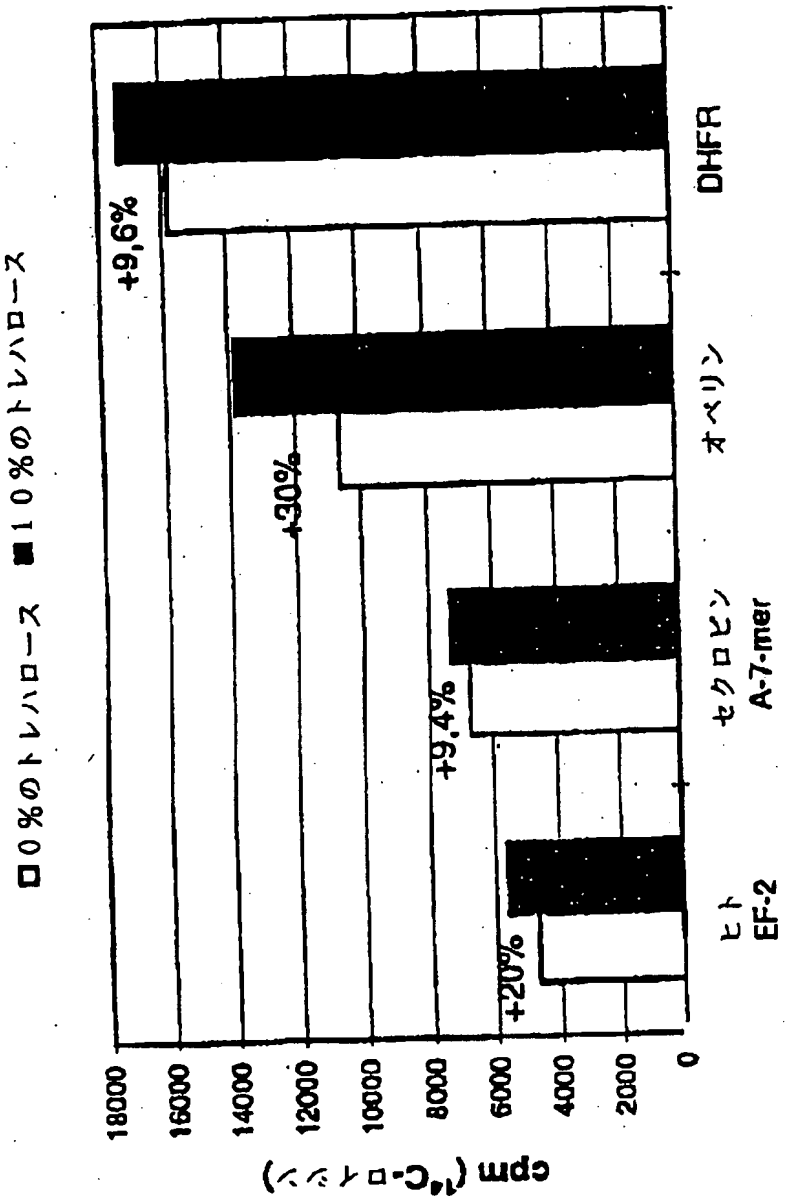


図2b)

[Drawing 3]

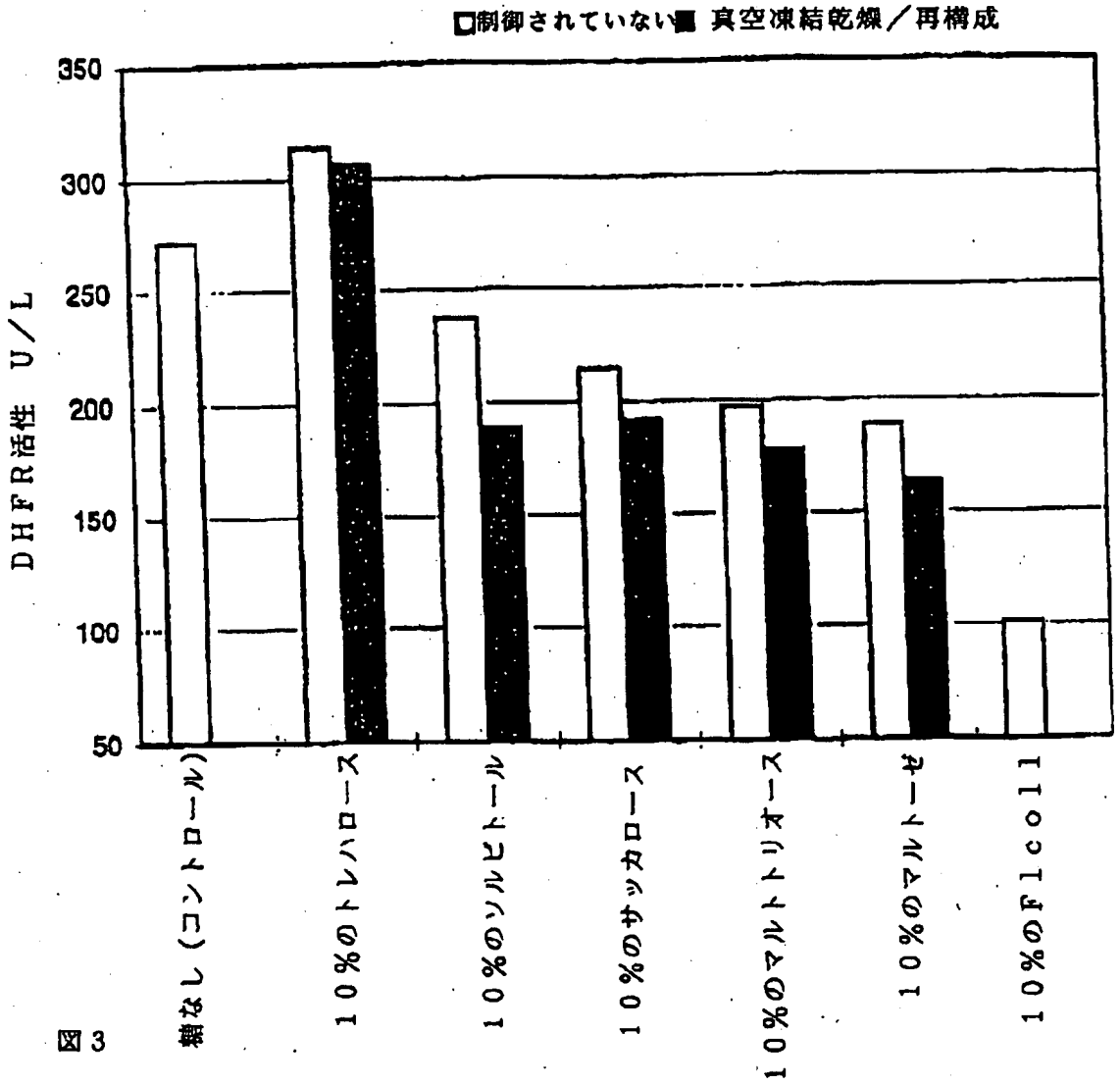

□制御されていない■ 真空凍結乾燥／再構成
DHFR活性 U／L
350
300
250
200
150
100
50
糖なし（コントロール）
１０％のトレハロース
１０％のソルビトール
１０％のサッカロース
１０％のマルトトリオース
１０％のマルトーゼ
１０％のＦｌｃｏｌｌ

図 3

[Drawing 4]

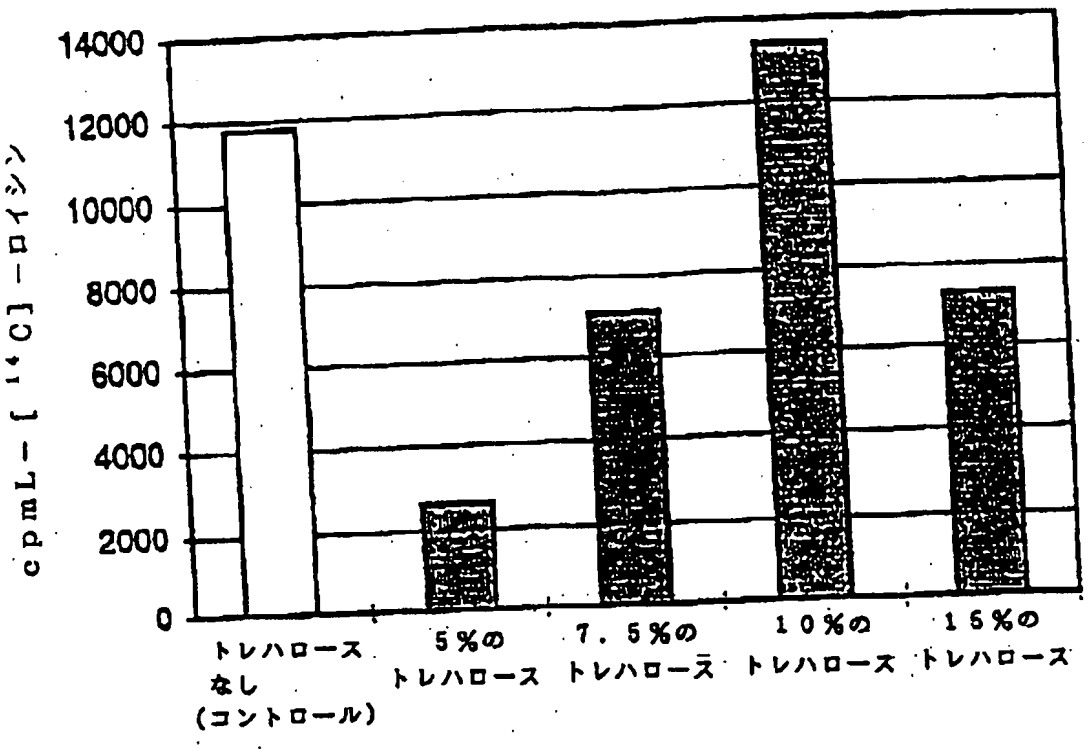
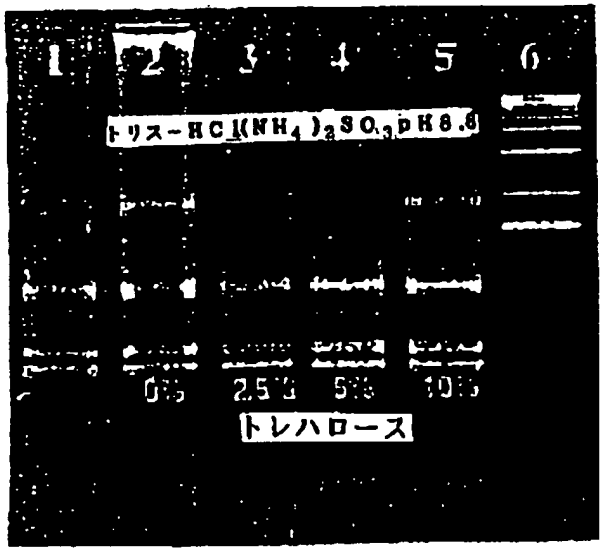
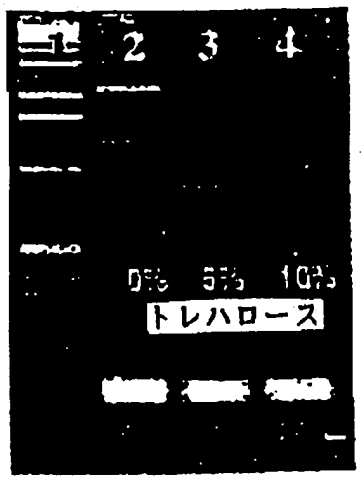

14000
12000
10000
8000
6000
4000
2000
0
cpmL－[14C]－ロイシン
トレハロースなし（コントロール）
5％のトレハロース
7．5％のトレハロース
10％のトレハロース
15％のトレハロース

図4

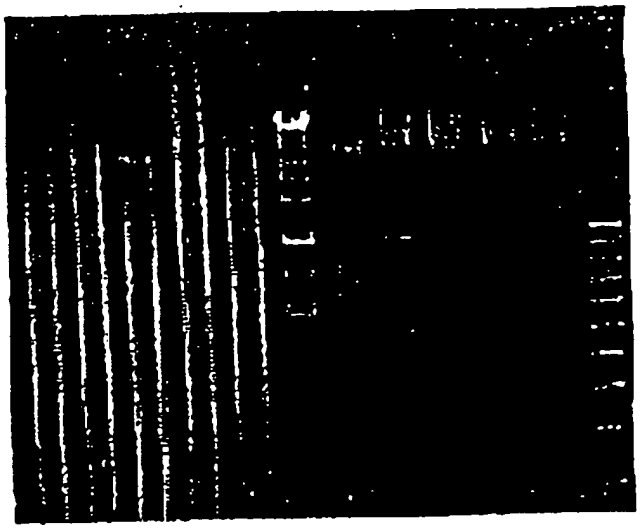
[Drawing 5]



5A



5B



5C

[Drawing 6]

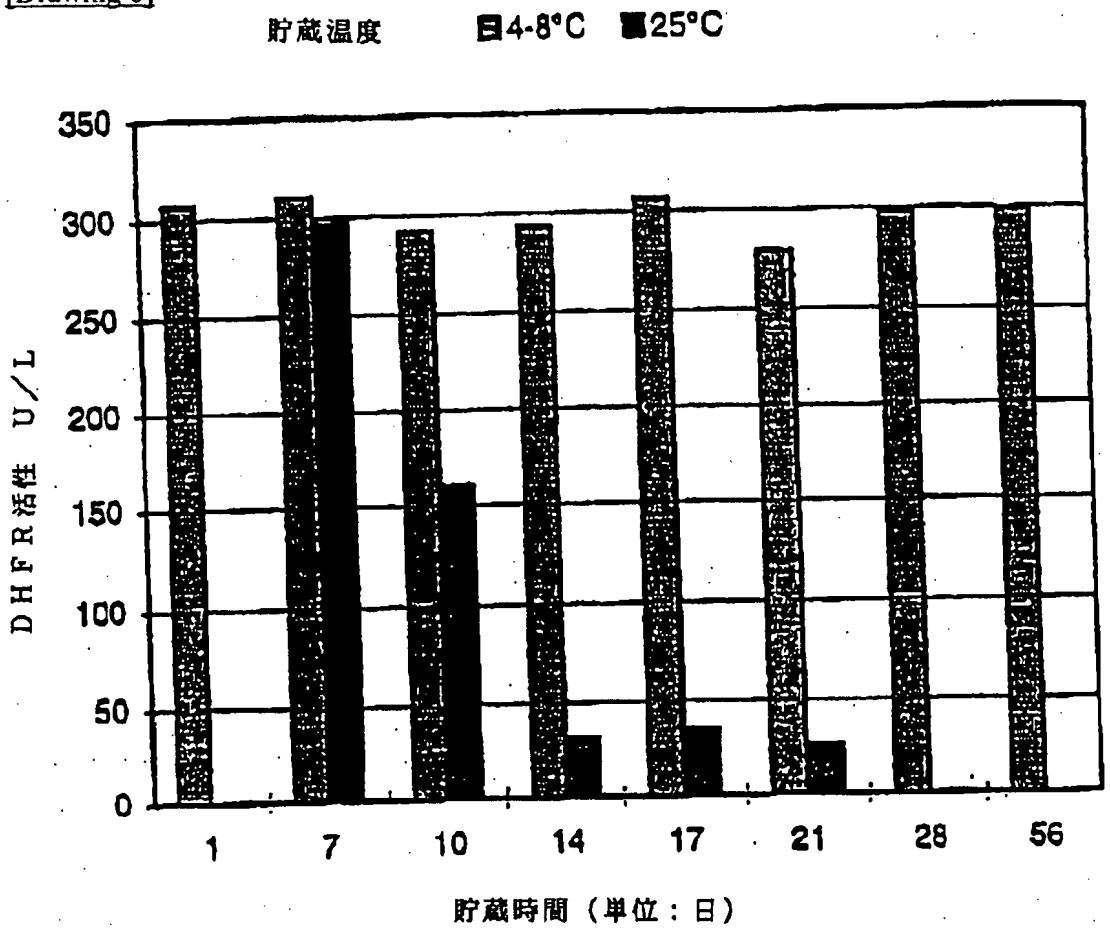


図 6

[Translation done.]